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EXPERIMENTAL ARTICLES

The Effect of Sodium Azide on the Thermotolerance of the Yeasts *Saccharomyces cerevisiae* and *Candida albicans*

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Abstract—The addition of sodium azide (a mitochondrial inhibitor) at a concentration of 0.15 mM to glucose-grown *Saccharomyces cerevisiae* or *Candida albicans* cells before exposing them to heat shock increased cell survival. At higher concentrations of azide, its protective effect on glucose-grown cells decreased. Furthermore, azide, even at low concentrations, diminished the thermotolerance of galactose-grown yeast cells. It is suggested that azide exerts a protective effect on the thermotolerance of yeast cells when their energy requirements are met by the fermentation of glucose. However, when cells obtain energy through respiratory metabolism, the azide inhibition of mitochondria enhances the damage inflicted on the cells by heat shock.

Key words: sodium azide, thermotolerance, alternative oxidase, mitochondria.

Sodium azide, a well-known inhibitor of cytochrome oxidase, blocks the mitochondrial respiratory chain between cytochromes *a* and a_3, so that cytochrome *a* is reduced, whereas cytochrome a_3 is oxidized [1]. Furthermore, azide inhibits the activity of mitochondrial F_1F_0-ATPase in the direction of ATP hydrolysis [2] and suppresses the activity of the antioxidant enzymes catalase [3], peroxidase [4], and superoxide dismutase [5].

Previously, we found that NaN_3 enhances the lethal effect of heat shock on the yeast *Debaryomyces vanriji* and exerts a considerable protective effect on the yeast *Saccharomyces cerevisiae* [6]. The opposite effects of azide were accounted yeast for by the presence of alternative oxidase in the former yeast and its absence in the latter.

It is known that alternative oxidase is located in the inner mitochondrial membrane and is insensitive to the action of azide, cyanide, and antimycin A, but is inhibited by hydroxamic acid derivatives [7]. Huh and Kang showed that the alternative oxidase of *Candida albicans* is encoded by the gene family *AOX1a*/*AOX1b* [8, 9]. The aim of the present work was to comparatively study the effects of azide on the thermotolerance of the *S. cerevisiae* strain Ψ-74-D694, the *C. albicans* strain CAI4, and its isogenic mutant strain WH324, defective in alternative oxidase.

MATERIALS AND METHODS

The *Saccharomyces cerevisiae* strain Ψ-74-D694 was obtained from S. Lindquist, the University of Chicago, United States, and the *Candida albicans* strains CAI4 (parent) and WH324 (mutant in alternative oxidase) from W.-K. Huh, the University of Seoul, South Korea. The strains were grown either in liquid YEPD medium containing (g/l) yeast extract, 5; peptone, 10; and glucose (dextrose), 20 or on solid YEPD medium containing, in addition to the above ingredients, 15 g/l agar.

Experiments were carried out with cells grown overnight at 30°C in liquid YEPD or YEPGal medium (in the latter medium, glucose was substituted by galactose in an equivalent amount). An aliquot of the overnight culture was inoculated into fresh medium and incubated for 4–5 h to the optical density OD = 0.4 (about 10^7 cells/ml).

The respiration rate of yeast cells was determined at 30°C using a Clark-type oxygen electrode with a temperature-controlled chamber containing 1.4 ml of yeast suspension. Sodium azide was added to a final concentration of 0.15 mM. To activate alternative oxidase, yeast cells were incubated in the presence of 10 μM antimycin A for 60 min. The respiration rate was expressed in nmol O_2/(min 10^7 cells) [10].

To study the effect of azide on the survival of cells exposed to heat shock, NaN_3 was added to 1-ml suspensions of *S. cerevisiae* and *C. albicans* cells in, respectively, YEPD and YEPGal media to final concentrations of 0.15, 1, and 2 mM. Then, the cell suspensions were exposed to heat shock at 45°C for certain periods of time, cooled, appropriately diluted, and plated onto agar YEPD medium. After incubation at 30°C for 24–48 h, the number of grown colonies was determined and the survival rate of yeast cells was calculated as the ratio of the number of colonies grown after heat shock to the number of colonies grown in the control. The ratio was expressed as a percentage.

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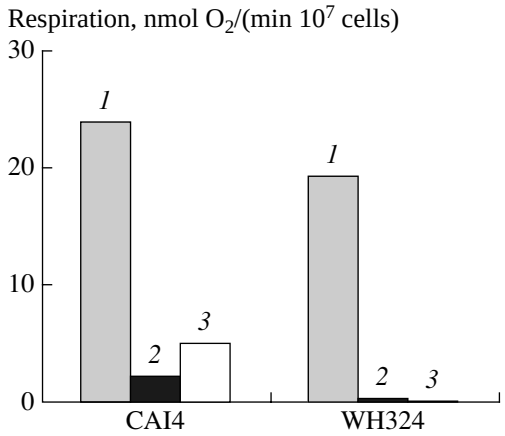

Fig. 1. The respiratory activity of glucose-grown cells of *C. albicans* strains CAI4 and WH324 (*1*) without inhibitors, (*2*) in the presence of 0.15 mM azide, and (*3*) after 1-h incubation in the presence of 10 μM antimycin A.

RESULTS

As shown earlier, the respiration of *S. cerevisiae* cells is completely inhibited by 0.15 mM sodium azide [6]. However, this concentration of azide inhibited the respiration of *D. vanriji* cells by only 72.3%, the residual respiration being completely inhibited by 2 mM benzohydroxamic acid [6]. In the present study, the respiration rate of *C. albicans* CAI4 cells grown in YEPD medium was found to be 23.9 and 2.1 nmol O_2/(min 10^7 cells) in the absence and in the presence of 0.15 mM azide, respectively (Fig. 1), which corresponded to a level of azide-resistant respiration equal to 8.8% of the total respiration. The respiratory activity of strain WH324, which is deficient in alternative oxidase, was lower (19.2 nmol O_2/(min 10^7 cells)) and almost fully sensitive to azide (Fig. 1).

It is known that alternative oxidase is induced in the presence of inhibitors of mitochondrial respiration [8, 9]. In our experiments, the incubation of *C. albicans* CAI4 and WH324 cells in YEPD medium in the presence of 10 μM antimycin A for 60 min led to the complete suppression of the respiration of WH324 cells, whereas the relative level of the azide-resistant respiration of CAI4 cells increased to 21.4% (Fig. 1). These data are indicative of the presence of alternative oxidase in *C. albicans* WH324 cells and its absence in *C. albicans* CAI4 cells [8].

In earlier experiments, NaN_3 at a concentration of 0.15 mM considerably augmented the thermotolerance of *S. cerevisiae* W303-1B cells grown in YEPD medium [6]. In the present study, this concentration of azide was found to increase the tolerance of another *S. cerevisiae* strain, Ψ-74-D694, to heat shock (Fig. 2a). However, higher concentrations of azide (1 and 2 mM) exerted a less pronounced protective effect. Similarly, 0.15 mM azide enhanced by about twofold the thermo-

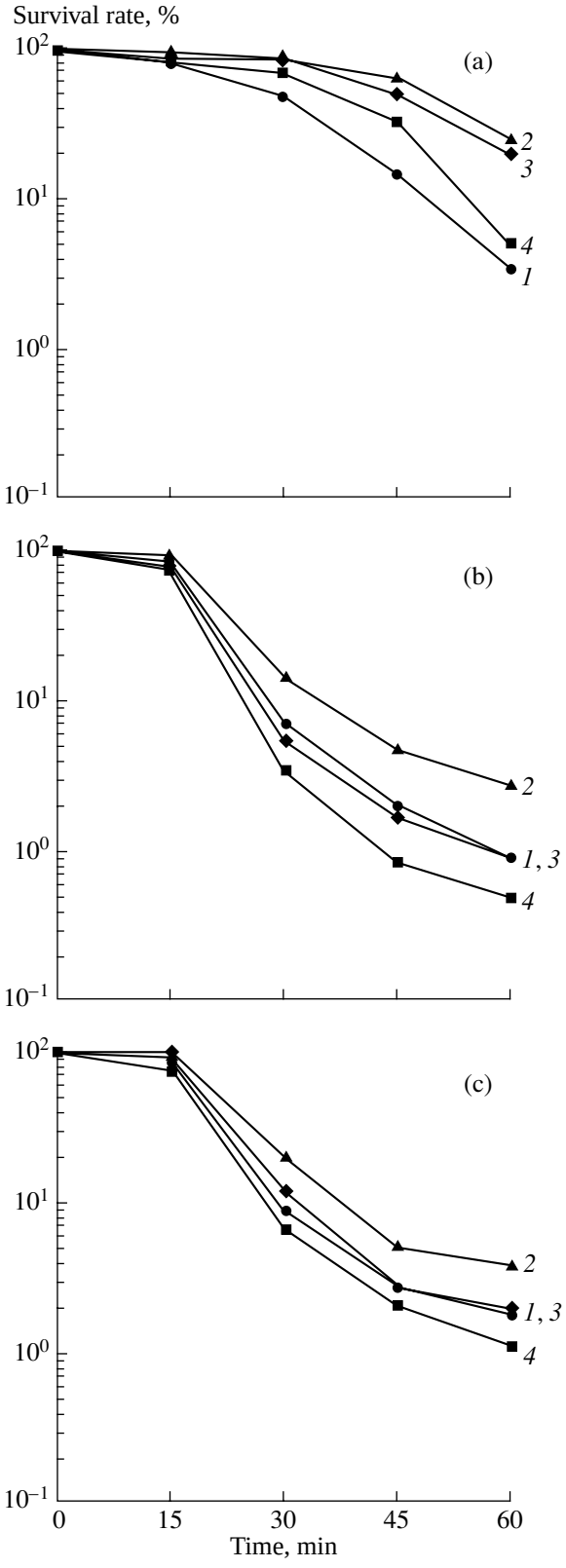

Fig. 2. The effect of azide at concentrations of (*1*) 0, (*2*) 0.15, (*3*) 1, and (*4*) 2 mM on the survival rate of glucose-grown cells of strains (a) *S. cerevisiae* Ψ-74-D694, (b) *C. albicans* CAI4, and (c) *C. albicans* WH324 exposed to heat shock (45°C).

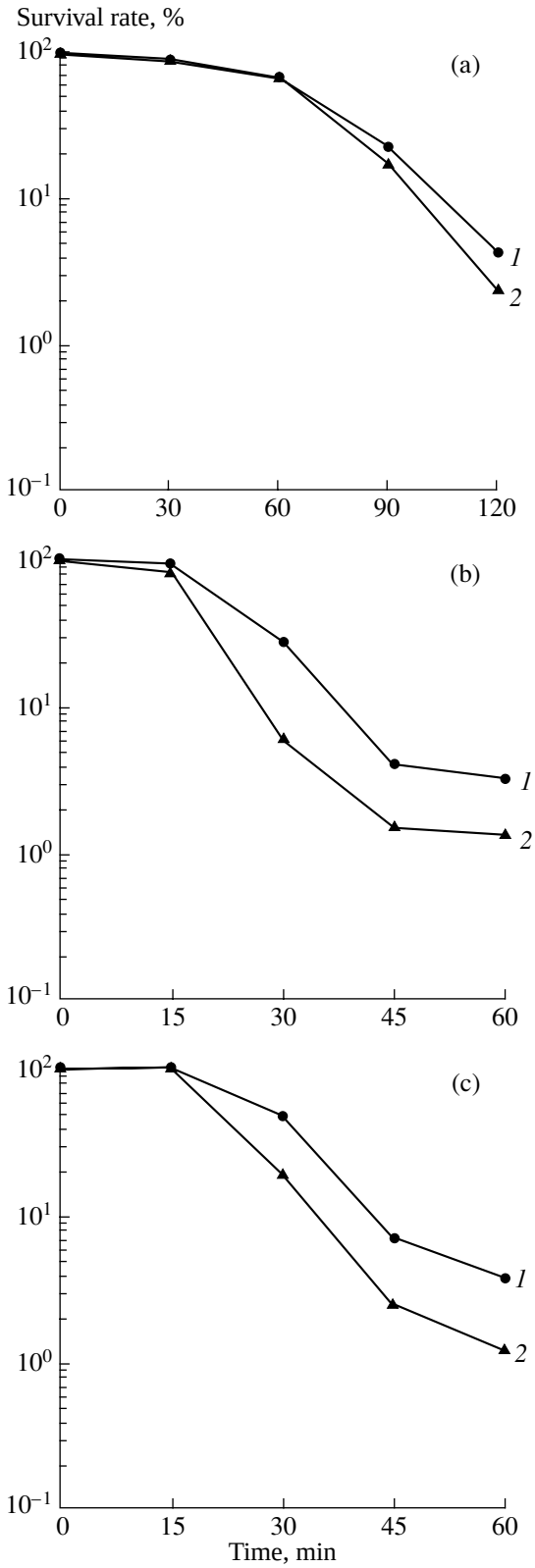

Fig. 3. The effect of azide at concentrations of (*1*) 0 and (*2*) 0.15 mM on the survival rate of galactose-grown cells of strains (a) *S. cerevisiae* Ψ-74-D694, (b) *C. albicans* CAI4, and (c) *C. albicans* WH324 exposed to heat shock (45°C).

tolerance of the glucose-grown parent strain *C. albicans* CAI4 (Fig. 2b) and its mutant WH324 defective in alternative oxidase (Fig. 2c), the protective effect being diminished to zero in the presence of 1 mM azide (curves *3*). At a still higher concentration (2 mM), azide decreased the thermotolerance of both *C. albicans* strains (Figs. 2b, 2c, curves *4*).

Thus, in contrast to the case of *D. vanriji*, 0.15 mM azide enhanced the thermotolerance of *S. cerevisiae* and *C. albicans* cells grown on glucose, irrespective of whether alternative oxidase was present or absent in these cells.

In the next set of experiments, we investigated the effect of azide on the thermotolerance of *S. cerevisiae* and *C. albicans* cells grown on galactose, which provides for a higher level of respiratory activity than glucose [11]. In agreement with the data of Sanchez *et al.* [12], *S. cerevisiae* cells grown on galactose turned out to be more thermoresistant than those grown on glucose (Figs. 2a, 3a). The same was true for *C. albicans* cells (Figs. 3b, 3c), although the difference between the thermotolerance of glucose- and galactose-grown cells of this species was not so significant (Figs. 2b, 2c).

The addition of 0.15 mM azide decreased the thermotolerance of galactose-grown cells of all three yeast strains studied (Fig. 3), the sensitizing effect of azide being most pronounced for the *C. albicans* strains CAI4 and WH324 (Figs. 3b, 3c). The azide-induced decrease in the thermotolerance of *S. cerevisiae* Ψ-74-D694 cells was lower and could be reliably detected only after an extended period (about 120 min) of incubation at 45°C (Fig. 3a).

DISCUSSION

Thus, azide differently affects the thermotolerance of *D. vanriji*, *C. albicans*, and *S. cerevisiae* cells. At a concentration of 0.15 mM, azide decreases the thermotolerance of glucose-grown *D. vanriji* cells [6] but enhances the thermotolerance of glucose-grown *S. cerevisiae* and *C. albicans* cells. The decrease in the protective effect of higher concentrations of azide can be explained by their inhibitory action on antioxidant enzymes, which may also be involved in the resistance of yeast cells to heat shock [13]. The thermotolerance of galactose-grown cells of both *C. albicans* strains, CAI4 and WH324, and the *S. cerevisiae* strain Ψ-74-D694 diminished in the presence of 0.15 mM azide. (It should be noted that the effect of azide on the galactose-grown *S. cerevisiae* cells is strain-specific. This phenomenon will be investigated by us in further studies.)

The data presented indicate that the protective effect of azide on cells exposed to heat shock does not depend on the functioning of alternative oxidase, since both the parent strain *C. albicans* CAI4 and its mutant strain defective in alternative oxidase, WH324, respond similarly to azide. At the same time, the different effects of azide on the survival of *S. cerevisiae* and *C. albicans*

cells grown on glucose and galactose suggests that there is a correlation between the respiratory activity of mitochondria and the thermotolerance of yeast cells.

The yeast *D. vanriji* cannot ferment glucose [14] and meets its energy requirements during growth on glucose only through the functioning of mitochondria. In contrast, the yeasts *S. cerevisiae* and *C. albicans* can actively ferment glucose [15] but only slightly ferment galactose [11, 16]. Therefore, mitochondria are the major source of energy (ATP) in the galactose-grown cells of *S. cerevisiae* and *C. albicans.* The data presented in the current paper strongly suggest that azide exerts protective effect on cells that obtain energy via glycolysis. However, if cells derive energy through the functioning of mitochondria, azide enhances the damaging effect of heat shock.

ACKNOWLEDGMENT

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